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(FILE 'HOME' ENTERED AT 12:29:59 ON 04 FEB 2002)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICONF'
ENTERED AT 12:31:45 ON 04 FEB 2002
E GRAHAM M?
E GRAHAM MIXHAEL?
E GRAHAM MIXHAEL?/AU
E GRAHAM MICHAEL?/AU
L1 5 S E2
L2 5 DUP REM L1 (0 DUPLICATES REMOVED)
L3 5 SORT L2 PY

=> d an ti so au ab pi l3 1-5

L3 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2002 ACS
AN 1997:34084 CAPLUS
DN 126:55944
TI Carnation genetic engineering to reduce expression of ACC synthase and ACC oxidase enzymes of ethylene biosynthetic pathway prolongs flower post-harvest life
SO PCT Int. Appl., 98 pp.
CODEN: PIXXD2
IN Michael, Michael Zenon; **Graham, Michael Wayne**; Cornish, Edwina Cecily; Gutterson, Neal Ira; Tucker, William Tinsley
AB The present invention relates generally to transgenic plants which exhibit prolonged post-harvest life properties. More particularly, the present invention is directed to transgenic carnation plants modified to reduce expression of one or more enzymes assocd. with the ethylene biosynthetic pathway. Flowers of such carnation plants do not produce ethylene, or produce ethylene in reduced amts., and are, therefore, capable of surviving longer post-harvest than flowers of non-genetically modified, naturally-occurring carnation plants.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9635792	A1	19961114	WO 1996-AU286	19960509
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN				
AU 9654930	A1	19961129	AU 1996-54930	19960509
AU 703841	B2	19990401		
EP 824591	A1	19980225	EP 1996-911869	19960509
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI				
JP 11504815	T2	19990511	JP 1996-533608	19960509

L3 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2002 ACS
AN 1997:679171 CAPLUS
DN 127:327456
TI Regulated excision of a target gene from the transformation vector in the recipient cell using a site-specific recombinase
SO PCT Int. Appl., 85 pp.
CODEN: PIXXD2
IN Surin, Brian Peter; De Feyter, Robert Charles; **Graham, Michael Wayne**; Waterhouse, Peter Michael; Keese, Paul Konrad; Shahjahan, Ali
AB A method of site-specific excision of a target gene from a transformation vector using a site-specific recombinase is described. This allows the transformation of the target organism with the removal of a selectable marker carried by the vector. Excision can be regulated or constitutive depending upon the promoter regulating the recombinase gene. As a result the same selectable marker can be used can be used in a no. of sequential transformations. The method can be generally used to regulate transgene expression in genetically-manipulated organisms, for example to promote

differentiation, de-differentiation, or any unidirectional developmental shift of a target cell which requires the time-specific expression of a particular gene. The method is particularly suited to the promotion of specific organogeneses in plants using organogenesis-promoting transgenes, wherein the organs which subsequently develop in said plants are genetically transformed with a desired gene but lack organogenesis-promoting transgenes. The use flp/frt and cre/loxP recombination systems in tobacco (Nicotiana plumbaginifolia) is demonstrated.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9737012	A1	19971009	WO 1997-AU197	19970327
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2250111	AA	19971009	CA 1997-2250111	19970327
AU 9721437	A1	19971022	AU 1997-21437	19970327
AU 717267	B2	20000323		
EP 922097	A1	19990616	EP 1997-913984	19970327
R: BE, CH, DE, ES, FR, GB, IT, LI, NL, SE				
JP 2000507446	T2	20000620	JP 1997-534743	19970327

L3 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2002 ACS
AN 1999:673015 CAPLUS
DN 131:307674
TI Reducing the phenotypic expression of a gene in plant using sense and antisense constructs
SO PCT Int. Appl., 82 pp.
CODEN: PIXXD2
IN Waterhouse, Peter Michael; Wang, Ming-Bo; **Graham, Michael Wayne**
AB Methods and means are provided for reducing the phenotypic expression of a gene of interest in plant cells, by introducing genetic constructs encoding sense and antisense RNA mols. directed towards the target gene. The RNA is capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base-pairing between the regions with sense and antisense nucleotide sequence such that at least the 10 consecutive nucleotides of the sense sequence base pair with the 10 consecutive nucleotides of the antisense sequence resulting in an artificial hairpin structure. The methods are directed towards reducing viral infection, resulting in extreme virus resistance. In another embodiment the methods are directed towards reducing the phenotypic expression of an endogenous gene in a plant cell.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9953050	A1	19991021	WO 1999-IB606	19990407
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9929514	A1	19991101	AU 1999-29514	19990407
EP 1068311	A1	20010117	EP 1999-910592	19990407
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

L3 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2002 ACS
AN 1999:626321 CAPLUS

Print selected from Online session

DN 131:238848
TI Control of gene expression by synthetic genes comprising multiple copies of repeat sequences
SO PCT Int. Appl., 161 pp.
CODEN: PIXXD2
IN **Graham, Michael Wayne**; Rice, Robert Norman
AB The present invention relates generally to a method of modifying gene expression and to synthetic genes for modifying endogenous gene expression in a cell, tissue or organ of a transgenic organism, in particular a transgenic animal or plant. More particularly, the present invention utilizes recombinant DNA technol. to post-transcriptionally modify or modulate the expression of a target gene in a cell, tissue, organ or whole organism, thereby producing novel phenotypes. Novel synthetic genes and genetic constructs which are capable or repressing delaying or otherwise reducing the expression of an endogenous gene or a target gene in an organism when introduced thereto are also provided. The synthetic gene comprises tandem inverted and/or direct repeats of a genetic sequence that is endogenous to the genome of the cell, tissue, organ or organism or which is derived from a non-endogenous gene. Such genetic constructs are exemplified by (1) bovine enterovirus RNA-dependent RNA polymerase gene sequences linked to the CMV promoter and/or the SV40L promoter, (2) porcine .alpha.-1,3-galactosyltransferase (Galt) gene operably linked to the CMV promoter and/or SV40L promoter, (3) and potato Y virus Nia gene operably linked to the 35S promoter and/or the sugarcane bacilliform virus promoter. These constructs can inactivate virus gene or Galt expression in animal cells or induce virus resistance in transgenic plants.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9949029	A1	19990930	WO 1999-AU195	19990319
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9929163	A1	19991018	AU 1999-29163	19990319
BR 9908967	A	20001219	BR 1999-8967	19990319
EP 1071762	A1	20010131	EP 1999-910039	19990319
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
GB 2353282	A1	20010221	GB 2000-24727	19990319

L3 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2002 ACS
AN 2001:713532 CAPLUS
DN 135:268121
TI Post-transcriptional gene silencing via reduction of a target transcript translation for manipulation in the phenotype of an animal
SO PCT Int. Appl., 176 pp.
CODEN: PIXXD2
IN **Graham, Michael Wayne**; Rice, Robert Norman; Murphy, Kathleen Margaret; Reed, Kenneth Clifford
AB The present invention relates generally to a method of inducing, promoting or otherwise facilitating a change in the phenotype of an animal cell or group of animal cells including a animal comprising said cells. The modulation of phenotypic expression is conveniently accomplished via genotypic manipulation through such means as reducing translation of a target transcript (co-suppression). One aspect of the present invention provides a genetic construct comprising a nucleotide sequence substantially identical to a target endogenous gene of a vertebrate animal cell, and further comprises a nucleotide sequence complementary to said target gene, wherein the sequences identical and complementary to said target gene are sepd. by an intron sequence. In prefered embodiment said

intron sequence is an intron from a gene encoding .beta.-globin, and even more preferred the .beta.-globin intron is human .beta.-globin intron 2. The ability to induce, promote or otherwise facilitate the silencing of expressible genetic sequences provides a means for modulating the phenotype in, for example, the medical or veterinary industries. Expressible genetic sequences contemplated by the present invention including not only genes normally resident in a particular animal cell (i.e. indigenous genes) but also genes introduced through recombinant means or through infection by pathogenic agents such as viruses.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001070949	A1	20010927	WO 2001-AU297	20010316

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

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L3 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2002 ACS
AN 1999:673015 CAPLUS
DN 131:307674
TI Reducing the phenotypic expression of a gene in plant using sense and antisense constructs
IN Waterhouse, Peter Michael; Wang, Ming-Bo; **Graham, Michael Wayne**
PA Commonwealth Scientific and Industrial Research Organisation, Australia
SO PCT Int. Appl., 82 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C12N015-11
ICS A01H003-00
CC 3-2 (Biochemical Genetics)
Section cross-reference(s): 10, 11
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9953050	A1	19991021	WO 1999-IB606	19990407
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9929514	A1	19991101	AU 1999-29514	19990407
EP 1068311	A1	20010117	EP 1999-910592	19990407
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
PRAI US 1998-56767	A	19980408		
US 1998-127735	A	19980803		
WO 1999-IB606	W	19990407		

AB Methods and means are provided for reducing the phenotypic expression of a gene of interest in plant cells, by introducing genetic constructs encoding sense and antisense RNA mols. directed towards the target gene. The RNA is capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base-pairing between the regions with sense and antisense nucleotide sequence such that at least the 10 consecutive nucleotides of the sense sequence base pair with the 10 consecutive nucleotides of the antisense sequence resulting in an artificial hairpin structure. The methods are directed towards reducing viral infection, resulting in extreme virus resistance. In another embodiment the methods are directed towards reducing the phenotypic expression of an endogenous gene in a plant cell.
ST plant gene expression sense antisense construct; virus disease resistance plant sense antisense vector; oil seed rape oleate content sense antisense vector
IT Promoter (genetic element)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(35S, in CoP construct; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Genetic vectors
(CoP (COmplimentary Pair), sense and antisense gene contg.; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Arabidopsis thaliana
Hazel (Corylus avellana)
(Fad2 gene from; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Gene, plant

RL: BPR (Biological process); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process); USES (Uses)
(Fad2, reduced expression of; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Gene, microbial
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(Nia, obtaining virus resistance using sense and antisense constructs of; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Stem-loop structure
(RNA capable of forming artificial; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Plant virus
(RNA, gene of interest from; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Promoter (genetic element)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(S4, in CoP construct; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Enhancer (genetic element)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(S7, in Co-P vector; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Rape (plant)
(desaturase gene from; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Gene
(expression, reducing of; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Potato virus Y
(gene Nia from; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Plant (Embryophyta)
(gene of interest from; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Conformation
(hairpin loop, artificial; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Repetitive DNA
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(inverted, perfect, generated from sense and antisense gene; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Viral RNA sequences
(of Nea gene from potato virus Y, and promoter regions, used in CoP vectors; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT DNA sequences
(of Nea-CoP and Fad2-CoP vectors, and Fad2 gene from Arabidopsis thaliana and Corylus avellana; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Fatty acids, preparation
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(profile, modifying of; reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Breeding, plant
Flaveria trinervia
Genetic engineering
Molecular cloning
(reducing phenotypic expression of gene in plant using sense and antisense constructs)

IT Flax
(rust resistance gene from; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Gene, plant
RL: BPR (Biological process); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(rust resistance, in sense and antisense orientation; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Promoter (genetic element)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(seed specific, use in Fad2-CoP vector; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Double stranded RNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(sense and antisense RNA mol. capable of forming; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Genetic element
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(spacer, between sense and antisense sequences; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Genetic element
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(terminator, nos, use in CoP vector; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Johnsongrass mosaic virus
(use of 5'-UTR region from; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Antisense RNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(use of chimeric genes encoding sense and; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Transgene
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(use of sense and antisense sequences of; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Viral RNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(use of; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT Disease resistance, plant
(viral; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT 9027-40-1, Pyruvate orthophosphate dikinase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(gene for; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT 112-80-1P, 9-Octadecenoic acid (9Z)-, preparation
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(in oilseed rape, increasing content of; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT 247211-72-9, DNA (potato virus Y gene Nia fragment)
RL: AGR (Agricultural use); BPR (Biological process); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process); USES (Uses)
(nucleotide sequence; reducing phenotypic expression of gene in plant using sense and antisense constructs)

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IT 152410-16-7, GenBank L26296 196217-79-5, GenBank A65102
RL: AGR (Agricultural use); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(nucleotide sequence; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT 247211-73-0, DNA (sinthetic genetic vector Gusd-CoP) 247211-74-1D, modified 247211-75-2 247211-76-3 247211-78-5 247211-80-9
RL: BPR (Biological process); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process); USES (Uses)
(nucleotide sequence; reducing phenotypic expression of gene in plant using sense and antisense constructs)
IT 103843-28-3, Desaturase
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(.delta.12, use of fad2 gene for; reducing phenotypic expression of gene in plant using sense and antisense constructs)
RE.CNT 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Calgene Inc; EP 240208 1997 CAPLUS
(2) Debonte; US 5850026 1998 CAPLUS
(3) Deutches Krebsforschungszentrum Stiftung Des Offentlichen Rechts; WO 98/05770 1998 CAPLUS
(4) Fire, A; Nature 1998, V391, P806 CAPLUS
(5) Jorgensen; US 5231020 1993 CAPLUS
(6) Jorgensen; US 5283184 1994 CAPLUS
(7) Jorgensen, R; Trends in Genetics 1999, V15(1), P11 CAPLUS
(8) Metzlaff, M; Cell 1997, V88, P845 CAPLUS
(9) Montgomery, M; Trends in Genetics 1988, V14(7), P255
(10) National Research Council of Canada; AU 20891/97 A 1997
(11) Nellen, W; Trends in biochemical sciences 1993, V18(11), P419 CAPLUS
(12) Stam, M; Annals of Botany 1997, V79, P3 CAPLUS
(13) The Research Foundation of State University of New York; EP 467349 1992 CAPLUS
(14) United States Biochemical Corporation; WO 92/13070 1992 CAPLUS
(15) Waterhouse, P; Proc Natl Acad Sci USA 1998, V95, P13959 CAPLUS
(16) Zeneca Limited; WO 93/23551 1993 CAPLUS

L3 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2002 ACS
AN 2001:713532 CAPLUS
DN 135:268121
TI Post-transcriptional gene silencing via reduction of a target transcript translation for manipulation in the phenotype of an animal
IN **Graham, Michael Wayne**; Rice, Robert Norman; Murphy, Kathleen Margaret; Reed, Kenneth Clifford
PA Benitec Australia Ltd., Australia; State of Queensland Through Its Department of Primary Industries
SO PCT Int. Appl., 176 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C12N015-11
ICS C12N015-63
CC 3-1 (Biochemical Genetics)
Section cross-reference(s): 6, 12, 13
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001070949	A1	20010927	WO 2001-AU297	20010316

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,

DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI AU 2000-6363 A 20000317
AU 2001-2700 A 20010124

AB The present invention relates generally to a method of inducing, promoting or otherwise facilitating a change in the phenotype of an animal cell or group of animal cells including a animal comprising said cells. The modulation of phenotypic expression is conveniently accomplished via genotypic manipulation through such means as reducing translation of a target transcript (co-suppression). One aspect of the present invention provides a genetic construct comprising a nucleotide sequence substantially identical to a target endogenous gene of a vertebrate animal cell, and further comprises a nucleotide sequence complementary to said target gene, wherein the sequences identical and complementary to said target gene are sepd. by an intron sequence. In prefered embodiment said intron sequence is an intron from a gene encoding .beta.-globin, and even more preferred the .beta.-globin intron is human .beta.-globin intron 2. The ability to induce, promote or otherwise facilitate the silencing of expressible genetic sequences provides a means for modulating the phenotype in, for example, the medical or veterinary industries. Expressible genetic sequences contemplated by the present invention including not only genes normally resident in a particular animal cell (i.e. indigenous genes) but also genes introduced through recombinant means or through infection by pathogenic agents such as viruses.

ST gene silencing posttranscriptional animal phenotype manipulation; transcript translation redn gene silencing

IT Plasmid vectors
(TOPO.BG12, human .beta.-globin intron 2 contg.; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(TP53, co-suppression, in murine cells in vitro; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(YB-1, co-suppression, in murine cells in vitro; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(c-erbB2, co-suppression, in vitro; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Bovine enterovirus
(co-suppression of RNA polymerase gene of; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Genetic vectors
(comprising sequences identical and complimentary to target gene, sepd. by intron; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Translation, genetic
(down-regulation of; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(for Brn-2 transcription factor, co-suppression, in melanoma cells in vitro; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT mRNA
RL: ANT (Analyte); BPR (Biological process); ANST (Analytical study); BIOL (Biological study); PROC (Process)
(for target endogenous gene or transgene; post-transcriptional gene

silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Proteins, specific or class
RL: ARU (Analytical role, unclassified); BPN (Biosynthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation)
(green fluorescent, enhanced, plasmid pEGFP-N1 encoding, co-suppression of; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Gene therapy
(in vertebrate; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Primate
(including human, manipulating phenotype of; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Genetic element
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(intron, 2, of human .beta.-globin gene, identical and complimentary to target gene sequences in vector sepd. by; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Animal
(lab. test, manipulating phenotype of; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Bird (Aves)
Fish
Livestock
Mammal (Mammalia)
Mouse
Reptile
Vertebrate (Vertebrata)
(manipulating phenotype of; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Transcription, genetic
(no redn. in, run-on assays for; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Dot blot hybridization
(of transcripts; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Plasmid vectors
(pCMVpur.GFP.BG12.PFG, contains palindrome of EGFP gene that is interrupted by insertion of .beta.-globin intron; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Phenotypes
(post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Gene
(processes, post-transcriptional silencing; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT PCR (polymerase chain reaction)
(real time, in transcription run-on assay; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Transgene
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(target, silencing; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)

Print selected from Online Session

(target; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Animal cell
(vertebrate, gene silencing in; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT Globins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(.beta.-globin, vector comprising intron from human gene for; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT 9068-09-1, .alpha. galactosyl transferase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(GalT, co-suppression of gene for, in transgenic mouse; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT 9002-10-2P, tyrosinase
RL: ARU (Analytical role, unclassified); BPN (Biosynthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation)
(co-suppression of gene for, detected in melanocytes; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT 9002-06-6P, Thymidine kinase
RL: ARU (Analytical role, unclassified); BPN (Biosynthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation)
(co-suppression of gene for; post-transcriptional gene silencing via redn. of target transcript translation for manipulation in phenotype of animal)

IT 363240-78-2, 1: PN: WO0170949 SEQID: 1 unclaimed DNA 363240-79-3, 2: PN: WO0170949 SEQID: 2 unclaimed DNA 363240-80-6, 3: PN: WO0170949 SEQID: 3 unclaimed DNA 363240-81-7, 4: PN: WO0170949 SEQID: 4 unclaimed DNA 363240-82-8, 5: PN: WO0170949 SEQID: 5 unclaimed DNA 363240-83-9, 6: PN: WO0170949 SEQID: 6 unclaimed DNA 363240-84-0, 7: PN: WO0170949 SEQID: 7 unclaimed DNA 363240-85-1, 8: PN: WO0170949 SEQID: 8 unclaimed DNA 363240-86-2, 9: PN: WO0170949 SEQID: 9 unclaimed DNA 363240-87-3 363240-88-4 363240-89-5 363240-90-8 363240-91-9 363240-92-0 363240-93-1 363240-94-2 363240-95-3 363240-96-4 363240-97-5 363240-98-6 363240-99-7 363241-00-3 363241-01-4
RL: PRP (Properties)
(unclaimed nucleotide sequence; post-transcriptional gene silencing via redn. of a target transcript translation for manipulation in the phenotype of an animal)

RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

(1) Birchler; Current Opinion in Genetics & Development 2000, V10, P211 CAPLUS
(2) Cogoni; Current Opinion in Genetics & Development 2000, V10, P638 CAPLUS
(3) Marathe; Plant Molecular Biology 2000, V43, P295 CAPLUS
(4) Oates; Developmental Biology 2000, V224, P20 CAPLUS
(5) Putlitz; Antisense & Nucleic Acid Drug Development 1999, V9, P241 CAPLUS
(6) Tavernarakis; Nature Genetics 2000, V24, P180 CAPLUS
(7) Ui-Tei; FEBS 2000, V479, P79 CAPLUS
(8) Wargelius; Biochem Biophys Research Comm 1999, V263, P156 CAPLUS

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(FILE 'HOME' ENTERED AT 12:29:59 ON 04 FEB 2002)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICONF'
ENTERED AT 12:31:45 ON 04 FEB 2002
E GRAHAM M?
E GRAHAM MIXHAEL?
E GRAHAM MIXHAEL?/AU
E GRAHAM MICHAEL?/AU
L1 5 S E2
L2 5 DUP REM L1 (0 DUPLICATES REMOVED)
L3 5 SORT L2 PY
L4 511 S POST-TRANSCRIPTIONAL GENE SILENCING
L5 174 DUP REM L4 (337 DUPLICATES REMOVED)
L6 29 S L5 AND PY<=1998
L7 29 SORT L6 PY
L8 14 S L5 AND (ANTISEN? AND SENSE?)
L9 14 SORT L8 PY

=> d an ti so au ab pi l9 1-14

L9 ANSWER 1 OF 14 MEDLINE
AN 97134915 MEDLINE
TI RNA as a target and an initiator of **post-transcriptional gene silencing** in transgenic plants.
SO PLANT MOLECULAR BIOLOGY, (1996 Oct) 32 (1-2) 79-88. Ref: 56
Journal code: A6O; 9106343. ISSN: 0167-4412.
AU Baulcombe D C
AB **Post-transcriptional gene silencing** in transgenic plants is the manifestation of a mechanism that suppresses RNA accumulation in a sequence-specific manner. The target RNA species may be the products of transgenes, endogenous plant genes or viral RNAs. For an RNA to be a target it is necessary only that it has sequence homology to the **sense** RNA product of the transgene. There are three current hypotheses to account for the mechanism of **post transcriptional gene silencing**. These models all require production of an **antisense** RNA of the RNA targets to account for the specificity of the mechanism. There could be either direct transcription of the **antisense** RNA from the transgene, **antisense** RNA produced in response to over expression of the transgene or **antisense** RNA produced in response to the production of an aberrant **sense** RNA product of the transgene. To determine which of these models is correct it will be necessary to find out whether transgene methylation, which is frequently associated with the potential of transgenes to confer **post-transcriptional gene silencing**, is a cause or a consequence of the process.

L9 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2002 ACS
AN 1996:637985 CAPLUS
DN 125:294613
TI **Post-transcriptional gene silencing** in tomato
SO Mech. Appl. Gene Silencing, [Easter Sch. Agric. Sci.], 57th (1996), Meeting Date 1995, 105-117. Editor(s): Grierson, Donald; Lycett, Grantley W.; Tucker, Gregory A. Publisher: Nottingham University Press, Nottingham, UK.
CODEN: 63NBAT
AU Hamilton, A. J.; Brown, S.; Grierson, D.
AB **Sense** and **antisense** transgenes have been used to inhibit the expression of a no. of tomato genes including those encoding the enzymes ACC-oxidase and polygalacturonase. The dramatic redn. in the activity of these enzymes enhances the storage qualities of these fruit and so may be of considerable economic importance. Here the authors describe some of their work conducted in order to understand how such

homologous transgenes can reduce gene expression so efficiently, focusing on.

L9 ANSWER 3 OF 14 AGRICOLA
AN 1998:58811 AGRICOLA
TI Pathogen-derived resistance targeted against the negative-strand RNA of tobacco mosaic virus: RNA strand-specific gene silencing?
SO The Plant journal : for cell and molecular biology, Feb 1998. Vol. 13, No. 4. p. 537-546
Publisher: Oxford : Blackwell Sciences Ltd.
ISSN: 0960-7412
AU Marano, M.R.; Baulcombe, D.
AB Tobacco plants transformed with the open-reading frame (ORF) of tobacco mosaic virus strain U1 (TMV-U1) encoding a 54 kDa (54K) region of the viral replicase are resistant against TMV strain U1. These plants are not resistant against the crucifer strain of TMV or the heterologous virus, potato virus X (PVX). However, they are resistant against derivatives of PVX containing fragments of the 54K ORF inserted either in the **sense** or anti-**sense** orientation. The smallest fragment that was a target of the resistance mechanism was a 383 nucleotide region from the central part of the 54K ORF. A transient gene expression assay revealed that this central region was also the target of a **post-transcriptional gene silencing** mechanism. However, unlike other examples of gene silencing associated with virus resistance, the silencing was specific for the anti-**sense** rather than the coding strand of the target RNA. Based on these data the authors propose that the TMV resistance is due, at least in part, to a type of transgene silencing.

L9 ANSWER 4 OF 14 MEDLINE
AN 1999147078 MEDLINE
TI Activation of systemic acquired silencing by localised introduction of DNA.
SO CURRENT BIOLOGY, (1999 Jan 28) 9 (2) 59-66.
Journal code: B44; 9107782. ISSN: 0960-9822.
AU Palauqui J C; Balzergue S
AB BACKGROUND: In plants, **post-transcriptional gene silencing** results in RNA degradation after transcription. Among tobacco transformants carrying a nitrate reductase (Nia) construct under the control of the cauliflower mosaic virus 35S promoter (35S-Nia2), one class of transformants spontaneously triggers Nia **post-transcriptional gene silencing** (class II) whereas another class does not (class I). Non-silenced plants of both classes become silenced when grafted onto silenced stocks, indicating the existence of a systemic silencing signal. Graft-transmitted silencing is maintained in class II but not in class I plants when removed from silenced stocks, indicating similar requirements for spontaneous triggering and maintenance. RESULTS: Introduction of 35S-Nia2 DNA by the gene transfer method called biolistics led to localised acquired silencing (LAS) in bombarded leaves of wild-type, class I and class II plants, and to systemic acquired silencing (SAS) in class II plants. SAS occurred even if the targeted leaf was removed 2 days after bombardment, indicating that the systemic signal is produced, transmitted and amplified rapidly. SAS was activated by **sense**, **antisense** and promoterless Nia2 DNA constructs, indicating that transcription is not required although it does stimulate SAS. CONCLUSIONS: SAS was activated by biolistic introduction of promoterless constructs, indicating that the DNA itself is a potent activator of **post-transcriptional gene silencing**. The systemic silencing signal invaded the whole plant by cell-to-cell and long-distance propagation, and reamplification of the signal.

L9 ANSWER 5 OF 14 MEDLINE
AN 2000457908 MEDLINE
TI dsRNA-mediated gene silencing in cultured Drosophila cells: a tissue

culture model for the analysis of RNA interference.
SO GENE, (2000 Jul 11) 252 (1-2) 95-105.
Journal code: FOP; 7706761. ISSN: 0378-1119.
AU Caplen N J; Fleenor J; Fire A; Morgan R A
AB RNA interference (RNAi) is a form of **post-transcriptional gene silencing** that has been described in a number of plant, nematode, protozoan, and invertebrate species. RNAi is characterized by a number of features: induction by double stranded RNA (dsRNA), a high degree of specificity, remarkable potency and spread across cell boundaries, and a sustained down-regulation of the target gene. Previous studies of RNAi have examined this effect in whole organisms or in extracts thereof; we have now examined the induction of RNAi in tissue culture. A screen of mammalian cells from three different species showed no evidence for the specific down-regulation of gene expression by dsRNA. By contrast, RNAi was observed in Drosophila Schneider 2 (S2) cells. Green fluorescent protein (GFP) expression in S2 cells was inhibited in a dose-dependent manner by transfection of dsRNA corresponding to gfp when GFP was expressed either transiently or stably. This effect was structure- and sequence-specific in that: (1) little or no effect was seen when **antisense** (or **sense**) RNA was transfected; (2) an unrelated dsRNA did not reduce GFP expression; and (3) dsRNA corresponding to gfp had no effect on the expression of an unrelated target transgene. This invertebrate tissue culture model should allow facile assays for loss of function in a well-defined cellular system and facilitate further understanding of the mechanism of RNAi and the genes involved in this process.

L9 ANSWER 6 OF 14 MEDLINE
AN 2000409274 MEDLINE
TI High-efficiency silencing of a beta-glucuronidase gene in rice is correlated with repetitive transgene structure but is independent of DNA methylation.
SO PLANT MOLECULAR BIOLOGY, (2000 May) 43 (1) 67-82.
Journal code: A6O; 9106343. ISSN: 0167-4412.
AU Wang M B; Waterhouse P M
AB Two transgenic callus lines of rice, stably expressing a beta-glucuronidase (GUS) gene, were supertransformed with a set of constructs designed to silence the resident GUS gene. An inverted-repeat (i/r) GUS construct, designed to produce mRNA with self-complementarity, was much more effective than simple **sense** and **antisense** constructs at inducing silencing. Supertransforming rice calluses with a direct-repeat (d/r) construct, although not as effective as those with the i/r construct, was also substantially more effective in silencing the resident GUS gene than the simple **sense** and **antisense** constructs. DNA hybridisation analyses revealed that every callus line supertransformed with either simple **sense** or **antisense** constructs, and subsequently showing GUS silencing, had the silence-inducing transgenes integrated into the plant genome in inverted-repeat configurations. The silenced lines containing i/r and d/r constructs did not necessarily have inverted-repeat T-DNA insertions. There was significant methylation of the GUS sequences in most of the silenced lines but not in the unsilenced lines. However, demethylation treatment of silenced lines with 5-azacytidine did not reverse the **post-transcriptional gene silencing** (PTGS) of GUS. Whereas the levels of RNA specific to the resident GUS gene were uniformly low in the silenced lines, RNA specific to the inducer transgenes accumulated to a substantial level, and the majority of the i/r RNA was unpolyadenylated. Altogether, these results suggest that both **sense**- and **antisense**-mediated gene suppression share a similar molecular basis, that unpolyadenylated RNA plays an important role in PTGS, and that methylation is not essential for PTGS.

L9 ANSWER 7 OF 14 MEDLINE
AN 2000216075 MEDLINE
TI Transgenic resistance to PVY(O) associated with post-transcriptional

silencing of P1 transgene is overcome by PVY(N) strains that carry highly homologous P1 sequences and recover transgene expression at infection.

SO MOLECULAR PLANT-MICROBE INTERACTIONS, (2000 Apr) 13 (4) 366-73.
Journal code: A9P; 9107902. ISSN: 0894-0282.

AU Maki-Valkama T; Valkonen J P; Kreuze J F; Pehu E

AB Resistance to Potato virus Y (PVY) has been obtained in our previous studies through expression of the PVY P1 gene in **sense** or **antisense** orientation in potato cv. Pito. In the present study, the mechanism and strain specificity of the resistance were analyzed. Several features including low steady-state P1 mRNA expression in the resistant P1 plants indicated that resistance was based on **post-transcriptional gene silencing** (PTGS).
Resistance was specific to PVY(O) isolates, the PVY strain group from which the P1 transgene was derived. However, according to group analyses, there was no distinguishing characteristic between the PVY(O) and PVY(N) strains P1 gene sequences. Therefore, the ability of the PVY(N) strains to overcome resistance could not be explained solely based on their P1 gene sequences. Infection with PVY(N) of the PVY(O)-resistant transgenic lines led to a recovery of expression of the P1 transgene. These data suggested that factors other than sequence homology are required in determination of the resistance specificity.

L9 ANSWER 8 OF 14 MEDLINE

AN 2000117527 MEDLINE

TI Distinct features of **post-transcriptional gene silencing** by **antisense** transgenes in single copy and inverted T-DNA repeat loci.

SO PLANT JOURNAL, (2000 Jan) 21 (1) 27-42.
Journal code: BRU; 9207397. ISSN: 0960-7412.

AU Stam M; de Bruin R; van Blokland R; van der Hoorn R A; Mol J N; Kooter J M

AB The application of **antisense** transgenes in plants is a powerful tool to inhibit gene expression. The underlying mechanism of this inhibition is still poorly understood. High levels of **antisense** RNA (as-RNA) are expected to result in strong silencing but often there is no clear correlation between as-RNA levels and the degree of silencing. To obtain insight into these puzzling observations, we have analyzed several petunia transformants of which the pigmentation gene chalcone synthase (Chs) is post-transcriptionally silenced in corollas by **antisense** (as) Chs transgenes. The transformants were examined with respect to the steady-state as-RNA level, transcription level of the as-transgenes, the repetitiveness and structure of the integrated T-DNAs, and the methylation status of the transgenes. This revealed that the transformants can be divided in two classes: the first class contains a single copy (S) T-DNA of which the as-Chs gene is transcribed, although several-fold lower than the endogenous Chs genes. As there are not sufficient as-RNAs to degrade every mRNA, we speculate that silencing is induced by double-stranded RNA. The second class contains two T-DNAs which are arranged as inverted repeats (IRs). These IR loci are severely methylated and the as-Chs transgenes transcriptionally barely active. The strongest silencing was observed with IR loci in which the as-Chs transgenes were proximal to the centre of the IR. Similar features have been described for co-suppression by IRs composed of **sense** Chs transgenes, suggesting that silencing by **antisense** IRs also occurs by co-suppression, either via ectopic DNA pairing or via dsRNA.

L9 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2002 ACS

AN 2000:718761 CAPLUS

DN 134:203186

TI Gene expression: Total silencing by intron-spliced hairpin RNAs

SO Nature (London) (2000), 407(6802), 319-320
CODEN: NATUAS; ISSN: 0028-0836

AU Smith, Neil A.; Singh, Surinder P.; Wang, Ming-Bo; Stoutjesdijk, Peter A.; Green, Allan G.; Waterhouse, Peter M.

AB **Post-transcriptional gene silencing** (PTGS), a sequence-specific RNA degrdn. mechanism inherent in many life

forms, can be induced in plants by transforming them with either **antisense** or co-suppression constructs, but typically this results in only a small proportion of silenced individuals. Here we show that gene constructs encoding intron-spliced RNA with a hairpin structure can induce PTGS with almost 100% efficiency when directed against viruses or endogenous genes. Using principles we developed for silencing constructs that express double-stranded RNA and inverted-repeat RNA, we made a construct encoding a single self complementary hairpin RNA (hpRNA) of the Niaprotease (Pro) gene sequence of potato virus Y (PVY). The construct contains **sense** and **antisense** Pro sequences flanking a nucleotide spacer fragment derived from uidaA (GUS) gene. About 60% of the plants that are transformed with the constructs were immune to the virus. In the next expt., we replaced the spacer with an intron sequence, which is spliced out during pre-mRNA processing to produce loopless hpRNA. As a control, the intron sequence was inserted in the reverse, non-splicing, orientation. When transformed into tobacco, 22 of 34 (65%) reverse-intron plants were immune, a similar frequency to plants transformed with the GUS spacer construct. Amazingly, 22 of 23 plants transformed with the construct contg. the functional intron were immune to the virus. This same enhancement was obsd. when hpRNA constructs against the endogenous .apprch.12-desaturase (Fad2) gene of Arabidopsis, in which 100% (30/30) of plants transformed with the intron construct showed silencing of the gene. The process of intron excision from the construct by spliceosome might help to align the complementary arms to the hairpin in an environment favoring RNA hybridization, promoting the formation of a duplex. Alternatively, splicing may transiently increase the amt. of hairpin RNA by facilitating, or retarding, the hairpin's passage from the nucleus, or by creating a smaller, less nuclease-sensitive loop.

L9 ANSWER 10 OF 14 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:311442 BIOSIS
TI Use of RNA interference (RNAi) to disrupt C-Kit gene expression in malignant human hematopoietic and neuroepithelial cells.
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 2, pp. 378b. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
AU Demir, Gokhan (1); Ptasznik, Andrzej; Zou, Shaomin; Fisher, Robert C.; Ratajczak, Mariusz Z.; Henningson, Carl; Gewirtz, Alan M.
AB In many cell types, **post-transcriptional gene silencing** (PTGS), or RNAi, is a highly reproducible strategy for disrupting gene expression at the mRNA level. The mechanism of RNAi remains uncertain, but the process is initiated by gene specific double stranded RNA (dsRNA) when introduced into a receptive cell. The utility of this technique in mammalian cells has been uncertain and was the focus of this study. To this end, we evaluated dsRNA effects on Kit receptor (KitR) expression in malignant human neuroepithelial and hematopoietic cells. cDNAs corresponding to 828 bp and 724 bp fragments respectively of KitR and green fluorescent protein (GFP) were cloned into pcDNA3 which was then utilized to in vitro transcribe **sense** and corresponding **antisense** RNA strands. After strand annealing, dsRNA integrity was confirmed by gel and then column purified. KitR expressing CHP 100 neuroepithelioma cells and HL-60 cells were employed as indicators. Lines were maintained in (RPMI+10% BCS) to which was added varying amounts (150-350 mug/ml) of Kit (KdsRNA) or GFP dsRNA (GdsRNA). Cells were incubated at 37degreeC, in 5% CO2 for 1-4 days, and then removed for FACS analysis of KitR expression using a monoclonal antibody (Dr. V. Brody, Univ. of Washington). No effect on KitR expression was observed until day 3, and then only in cells exposed to the KdsRNA. For example, after incubation with 150 mug/ml of KdsRNA, the percentage of (+) CHP cells decreased from 96+-2% to 80+-3%. The mean geometric fluorescence intensity (FI) on expressing cells decreased 2.25+0.25 fold ($p<0.01$). At a dose of 250 mug/ml of KdsRNA, KitR was decreased to 67+-2% and the FI decreased by 2.75+-0.50 fold ($p<0.01$). Doses >350 mug/ml of dsRNA were toxic. In HL 60

cells, KdsRNA doses < 280 mug/ml were ineffective, but at that dose the % of (+) cells decreased from 84+-2% to 36+-2%. However, FI decreased only 1.38+-0.5 fold. To further document KitR disruption, HL-60 cells were exposed to KdsRNA (300 mug/ml X 3 days) and then stimulated with SCF (150 ng/ml). Autophosphorylation of lyn, a known downstream effect of KitR engagement, was significantly diminished in KdsRNA treated cells compared to controls. We conclude that some mammalian cells are variably susceptible to RNAi, and thereby provide support for the development of therapeutically motivated PTGS in patients with malignant disease.

L9 ANSWER 11 OF 14 MEDLINE
AN 2001640922 MEDLINE
TI Transgene-mediated **post-transcriptional gene silencing** is inhibited by 3' non-coding sequences in Paramecium.
SO NUCLEIC ACIDS RESEARCH, (2001 Nov 1) 29 (21) 4387-94.
Journal code: 0411011. ISSN: 1362-4962.
AU Galvani A; Sperling L
AB Homology-dependent gene silencing is achieved in Paramecium by introduction of gene coding regions into the somatic nucleus at high copy number, resulting in reduced expression of all homologous genes. Although a powerful tool for functional analysis, the relationship of this phenomenon to gene silencing mechanisms in other organisms has remained obscure. We report here experiments using the T4a gene, a member of the trichoeyst matrix protein (TMP) multigene family encoding secretory proteins, and the ND7 gene, a single copy gene required for exocytotic membrane fusion. Silencing of either gene leads to an exocytosis-deficient phenotype easily scored on individual cells. For each gene we have tested the ability of different combinations of promoter, coding and 3' non-coding regions to provoke silencing, and analyzed transcription and steady-state RNA in the transformed cells. We provide evidence that homology-dependent gene silencing in Paramecium is post-transcriptional and that both **sense** and **antisense** RNA are transcribed from the transgenes, consistent with a role for dsRNA in triggering silencing. Constructs with and without promoters induce gene silencing. However, transgenes that contain 3' non-coding regions do not induce gene silencing, despite **antisense** RNA production. We present a model according to which different pathways of RNA metabolism compete for transcripts and propose that the relative efficiencies of dsRNA formation and of 3' RNA processing of **sense** transgene transcripts determine the outcome of transformation experiments.

L9 ANSWER 12 OF 14 MEDLINE
AN 2001509338 MEDLINE
TI Transgenic resistance in potato plants expressing potato leaf roll virus (PLRV) replicase gene sequences is RNA-mediated and suggests the involvement of **post-transcriptional gene silencing**.
SO ARCHIVES OF VIROLOGY, (2001 Jul) 146 (7) 1337-53.
Journal code: 8L7; 7506870. ISSN: 0304-8608.
AU Vazquez Rovere C; Asurmendi S; Hopp H E
AB Genetically engineered expression of replicase encoding sequences has been proposed as an efficient system to confer protection against virus diseases by eliciting protection mechanisms in the plant. Potato leaf-roll was one of the first diseases for which this kind of protection was engineered in potato plants. However, details of the protecting mechanism were not reported, so far. The ORF2b of an Argentinean strain of PLRV was cloned and sequenced finding 94% and 97% of homology with Australian and Dutch strains, respectively. To elucidate the mechanism of protection against PLRV infection, three versions of ORF2b (non-translatable **sense**, translatable **sense** with an engineered ATG and **antisense**) were constructed under the control of the 35S CaMV promoter and the nos terminator and introduced in potato plants (cv. Kennebec) by Agrobacterium tumefaciens-mediated transformation. Grafting infection experiments showed that resistant transgenic plants could be obtained with any of the constructs, suggesting that the mechanism of

protection is independent of the expression of protein and is RNA mediated. Field trial infection confirmed that resistant transgenic events were obtained. Biolistic transient transformation experiments of leaves derived from transgenic plants using a gene coding for the fusion protein GUS-ORF2b, followed by scoring of the number of GUS expressing leaf spots, supported that the protection is mediated by a **post-transcriptional gene silencing** mechanism.

L9 ANSWER 13 OF 14 SCISEARCH COPYRIGHT 2002 ISI (R)
AN 2002:27850 SCISEARCH
TI Graft transmission of induced and spontaneous post-transcriptional silencing of chitinase genes
SO PLANT JOURNAL, (DEC 2001) Vol. 28, No. 5, pp. 493-501.
Publisher: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND.
ISSN: 0960-7412.
AU Crete P; Leuenberger S; Iglesias V A; Suarez V; Schob H; Holtorf H; van Eeden S; Meins F (Reprint)
AB **Sense** and **antisense** tobacco chitinase (CHN) transgenes, Luciferase-CHN transcriptional fusions, and promoterless CHN cDNAs were introduced biolistically into CHN transformants of tobacco that never exhibit spontaneous gene silencing. All of the constructs tested induced systemic silencing of the resident CHN transgene and endogenes. Nuclear run-on transcription assays showed that local introduction of additional gene copies triggers systemic **post-transcriptional gene silencing** (PTGS).
Together, this provides evidence that additional transgene copies need not be either highly transcribed or produce **sense** transcripts to evoke production of systemic PTGS signals. CHN PTGS was transmitted by top grafting, but not by reciprocal grafting of mature stems or the exchange of tissue plugs. Thus, the commonly encountered difficulties in achieving graft-transmission could reflect the method used. Silencing in **sense** but not **antisense** transformants was transmitted by grafting to a high-expressing **sense** CHN scion suggesting that the elaboration of mobile signals may not be an essential feature of **antisense**-mediated gene silencing.

L9 ANSWER 14 OF 14 SCISEARCH COPYRIGHT 2002 ISI (R)
AN 2001:835727 SCISEARCH
TI PTGS in plants, a virus resistance mechanism
SO M S-MEDECINE SCIENCES, (AUG-SEP 2001) Vol. 17, No. 8-9, pp. 845-855.
Publisher: MASSON EDITEUR, 120 BLVD SAINT-GERMAIN, 75280 PARIS 06, FRANCE.
ISSN: 0767-0974.
AU Beclin C (Reprint); Vaucheret H
AB **Post-transcriptional gene silencing** (PTGS) in plants and quelling in fungi are transgene-induced silencing phenomena, resulting from the degradation of transgene RNAs and homologous endogenous RNAs. PTGS shows similarities with RNAi in animals, a phenomenon induced by injection of double-stranded RI A (dsRNA) or introduction of transgenes expressing dsRNA. First, PTGS and RNAi both involve dsRNA. Second, they can be dissected into three steps: localized initiation, propagation of a sequence-specific systemic signal, maintenance in silenced tissues. Finally, they both correlate with the accumulation of 25nt **sense** and anti-**sense** RNTAs.
Genetic dissection and cloning of genes regulating PTGS, quelling and RNAi confirmed the links between these three phenomena. Indeed, all three involve a putative RNA-dependent-INA polymerase and a protein similar to the translation initiator factor eIF2C. However some differences can be noticed. In particular, PTGS in plants requires two genes, SGS3 (encoding a protein of unknown function) and MET1 (encoding a DNA-methyltransferase), which are not required for RNAi Indeed, the genomes of C elegans and Drosophila (two organisms undergoing RNAi) lack both methylation and orthologs of the SGS3 gene). Several experiments revealed that PTGS is a general mechanism of virus resistance. In particular, we showed that Arabidopsis mutants impaired in PTGS are hypersensitive to

infection by the virus CMV. However, many viruses have developed strategies to counteract PTGS and therefore succeed to infect plants. Because viruses may act as targets, inducers or inhibitors of PTGS, the success and the extent of virus infection therefore depends on the competition between plant PTGS defenses and virus counteracting effects.

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L6 ANSWER 16 OF 142 MEDLINE
TI Gene targeting in normal somatic cells: inactivation of the interferon-gamma receptor in myoblasts.
SO NATURE GENETICS, (1994 Jan) 6 (1) 90-7.
Journal code: BRO. ISSN: 1061-4036.
AU Arbones M L; Austin H A; Capon D J; Greenburg G
AB Gene targeting in somatic cells represents a potentially powerful method for **gene therapy**, yet with the exception of pluripotent mouse embryonic stem (ES) cells, **homologous recombination** has not been reported for a well characterized, non-transformed mammalian cell. Applying a highly efficient strategy for targeting an integral membrane protein--the interferon gamma receptor--in ES cells, we have used **homologous recombination** to target a non-transformed somatic cell, the mouse myoblast, and to compare targeting efficiencies in these two cell types. Gene-targeted myoblasts display the properties of normal cells including normal morphology, ability to differentiate in vitro, stable diploid karyotype, inability to form colonies in soft agar and lack of tumorigenicity in nude mice.

L6 ANSWER 6 OF 142 MEDLINE

TI Fidelity of targeted recombination in human fibroblasts and murine embryonic stem cells.

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1991 Sep 15) 88 (18) 8067-71.
Journal code: PV3. ISSN: 0027-8424.

AU Zheng H; Hasty P; Brenneman M A; Grompe M; Gibbs R A; Wilson J H; Bradley A

AB Targeted recombination in murine embryonic stem cells promises to be a powerful tool for introducing specific mutations into target genes to study development in mice and to create animal models of human disease. Gene targeting also holds potential for correcting genetic defects as an approach to human **gene therapy**. To precisely modify target genes, **homologous recombination** must proceed with high fidelity. However, several results have suggested that targeted recombination may be highly mutagenic. To test the accuracy of gene targeting we analyzed 44 independent targeted recombinants at the hypoxanthine phosphoribosyltransferase (HPRT) locus in a human fibroblast cell line and in mouse embryonic stem cells. We surveyed 80 kilobases around the sites of recombination by using chemical cleavage of mismatches. Only two mutations were found: a T----G transversion and a thymidine deletion. Thus, gene targeting in mammalian cells can be extremely accurate. These results demonstrate the feasibility of generating precise modifications of mammalian genomes by gene targeting.

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.ches to human diseases.

L6 ANSWER 23 OF 142 MEDLINE
TI Gene targeting with a replication-defective adenovirus **vector**.
SO JOURNAL OF VIROLOGY, (1995 Oct) 69 (10) 6180-90.
Journal code: KCV. ISSN: 0022-538X.
AU Fujita A; Sakagami K; Kanegae Y; Saito I; Kobayashi I
AB Wide application of the gene-targeting technique has been hampered by its low level of efficiency. A replication-defective adenovirus **vector** was used for efficient delivery of donor DNA in order to bypass this problem. **Homologous recombination** was selected between a donor neo gene inserted in the adenovirus **vector** and a target mutant neo gene on a nuclear papillomavirus plasmid. These recombinant adenoviruses allowed gene transfer to 100% of the treated cells without impairing their viability. Homologous recombinants were obtained at a level of frequency much higher than that obtained by electroporation or a calcium phosphate procedure. The structure of the recombinants was analyzed in detail after recovery in an Escherichia coli strain. All of the recombinants examined had experienced a precise correction of the mutant neo gene. Some of them had a nonhomologous rearrangement of their sequences as well. One type of nonhomologous recombination took place at the end of the donor-target homology. The **vector** adenovirus DNA was inserted into some of the products obtained at a high multiplicity of infection. The insertion was at the end of the donor-target homology with a concomitant insertion of a 10-bp-long filler sequence in one of the recombinants. The possible relationship between these rearrangements and the **homologous recombination** is discussed. These results demonstrate the applicability of adenovirus-mediated gene delivery in gene targeting and **gene therapy**.

L6 ANSWER 37 OF 142 MEDLINE
TI Reversible immortalization of mammalian cells mediated by retroviral transfer and site-specific recombination.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Aug 20) 93 (17) 8971-6.
Journal code: PV3. ISSN: 0027-8424.
AU Westerman K A; Leboulch P
AB A procedure of reversible immortalization of primary cells was devised by retrovirus-mediated transfer of an oncogene that could be subsequently excised by site-specific recombination. This study focused on the early stages of immortalization: global induction of proliferation and life span extension of cell populations. Comparative analysis of Cre/LoxP and FLP/FRT recombination in this system indicated that only Cre/LoxP operates efficiently in primary cells. Pure populations of cells in which the oncogene is permanently excised were obtained, following differential selection of the cells. Cells reverted to their preimmortalized state, as indicated by changes in growth characteristics and p53 levels, and their fate conformed to the telomere hypothesis of replicative cell senescence. By permitting temporary and controlled expansion of primary cell populations without retaining the transferred oncogene, this strategy may facilitate **gene therapy** manipulations of cells unresponsive to exogenous growth factors and make practical gene targeting by **homologous recombination** in somatic cells. The combination of retroviral transfer and site-specific recombination should also extend gene expression studies to situations previously inaccessible to experimentation.

retrotransposons in the germline.

L5 ANSWER 6 OF 29 MEDLINE
AN 2000409274 MEDLINE
TI High-efficiency **silencing** of a beta-glucuronidase **gene** in rice is correlated with repetitive transgene structure but is independent of DNA methylation.
SO PLANT MOLECULAR BIOLOGY, (2000 May) 43 (1) 67-82. Journal code: 9106343. ISSN: 0167-4412.
AU Wang M B; Waterhouse P M
AB Two transgenic callus lines of rice, stably expressing a beta-glucuronidase (GUS) **gene**, were supertransformed with a set of constructs designed to **silence** the resident GUS **gene** . An inverted-**repeat** (i/r) GUS construct, designed to produce mRNA with self-complementarity, was much more effective than simple **sense** and antisense constructs at inducing **silencing**. Supertransforming rice calluses with a direct-**repeat** (d/r) construct, although not as effective as those with the i/r construct, was also substantially more effective in **silencing** the resident GUS **gene** than the simple **sense** and antisense constructs. DNA hybridisation analyses revealed that every callus line supertransformed with either simple **sense** or antisense constructs, and subsequently showing GUS **silencing**, had the **silence** -inducing transgenes integrated into the plant genome in inverted-**repeat** configurations. The **silenced** lines containing i/r and d/r constructs did not necessarily have inverted-**repeat** T-DNA insertions. There was significant methylation of the GUS sequences in most of the **silenced** lines but not in the unsilenced lines. However, demethylation treatment of **silenced** lines with 5-azacytidine did not reverse the post-transcriptional **gene** **silencing** (PTGS) of GUS. Whereas the levels of RNA specific to the resident GUS **gene** were uniformly low in the **silenced** lines, RNA specific to the inducer transgenes accumulated to a substantial level, and the majority of the i/r RNA was unpolyadenylated. Altogether, these results suggest that both **sense**- and antisense-mediated **gene** suppression share a similar molecular basis, that unpolyadenylated RNA plays an important role in PTGS, and that methylation is not essential for PTGS.